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Original Article

Topical imiquimod treatment for human papillomavirus infection in patients with and without cervical/vaginal intraepithelial neoplasia

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Abstract

Objective: To evaluate the efficacy and toxicity of topical imiquimod for the treatment of persistent human papillomavirus (HPV) infection in patients with or without cervical/vaginal intraepithelial neoplasia (CIN/VAIN).
Methods: Patients with persistent HPV infection (≥1 year) after a history of treatment for cervical or vaginal neoplasm but normal histology and cytology, abnormal Papanicolaou (Pap) smears without abnormal histology, and untreated histology-documented CIN/VAIN Grade 1/2/3 with HPV-positive testing were recruited. Patients were instructed to apply 250 mg of 5% imiquimod cream intravaginally on consecutive days or at least twice weekly on an outpatient basis for a minimum of 12 doses. A group of age- and previous diagnosis-matched, imiquimod-untreated historical controls ($n = 20$) were selected. The main outcome measures included HPV DNA detection, cytology, and colposcopy/histology at 6 months after treatment.
Results: A total of 72 patients were eligible for analysis. At a median follow-up of 33.6 months, 37 patients (51.4%) had cytological/histological regression and tested HPV-negative. Six patients (8.3%) had progressive cytology/histology with persistent HPV infections. Of the 72 treated patients, 26 patients who had a normal Pap test but were persistently HPV-positive for at least 1 year had a complete regression rate of 65.4%, which was significantly different from the rate (30%) observed in the untreated historical control ($p = 0.036$). Six patients with histologically proven CIN2/3 or VAIN2/3 had a complete regression rate of 66.6% (4/6).
Conclusions: The tolerability of intravaginal self-administered imiquimod is confirmed. Its efficacy in the treatment of women with persistent HPV infection and normal cytology warrants further randomized, controlled trials to determine appropriate dosages and scheduling.

Keywords: cervical intraepithelial neoplasia; human papillomavirus; imiquimod; vaginal intraepithelial neoplasia

Introduction

There is substantial epidemiological and molecular pathology evidence indicating that human papillomavirus (HPV) plays an etiologic role in cervical cancer and cervical intraepithelial neoplasia (CIN) [1]. The incorporation of HPV testing with cervical cytology in primary cervical cancer screening has been widely used [2]. However, identifying women with normal cytology and histology who are infected with high-risk HPV may result in substantial anxiety for these women [3]. HPV-related emotional distress has been noted. Therefore, a strategy to effectively manage persistent HPV infection or inflammation would have far-reaching global health and economic impact. HPV testing as triage for borderline or mild dyskaryosis and follow-up after conization

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has also been advocated [4−7]. However, the emergence of secondary neoplasia in the genital tract or relapse may occur more than 5 or 10 years later [8,9], and follow-up for women with persistent high-risk HPV infection can only be performed periodically.

As compared to CIN, studies on the natural history of vaginal intraepithelial neoplasia (VAIN) are relatively limited [10]. In addition, the relationship between persistent HPV infection and progression in vaginal neoplasia is still unclear [11−13]. In the management of CIN/VAIN, CIN1 can be observed within 2 years if it is nonprogressive while ≥CIN2 lesions are excised. A conservative management policy is generally applied to special populations, including adolescents and pregnant women [14]. VAIN may be difficult to manage, especially in the presence of multifocal lesions when the vaginal vault is involved after hysterectomy [15]. Surgical excision is the mainstay of treatment for high-grade VAIN, but various other modalities, including carbon dioxide laser therapy, 5-fluorouracil cream application, and cryotherapy have been proposed [15]. In a previous study of 132 women, eight cases progressed from high-grade VAIN to invasive cancer, whereas seven of the eight VAIN1 patients regressed during an observational approach after biopsy [16].

Treatment of CIN or VAIN after the failure of standard therapy is challenging. Imiquimod (5% cream) has been shown to be safe and effective in the treatment of genital warts caused by low-risk HPV infections [17,18]. Topical imiquimod has also been used for oral leukoplakia [19], basal cell carcinoma [20], vulvar intraepithelial neoplasia (VIN) [21,22], and VAIN [23−26]. The mechanism for the eradication of genital verrucous lesions with imiquimod may involve the induction of both innate and cellular immunity [18]. Antiviral activity may be stimulated through the induction of cytokines, such as interferon-α (IFN-α), tumor necrosis factor-α (TNF-α), and interleukins (ILs) [18]. It is known that imiquimod activates immune cells through the toll-like receptor 7 (TLR7), commonly involved in pathogen recognition, on the cell surface. Cells activated by imiquimod via TLR7 secrete cytokines such as IFN-α, IL-6, and TNF-α. There is evidence that imiquimod, when applied to skin, cervix, or vagina, can lead to the activation of naïve dendritic cells, which subsequently migrate to local lymph nodes to activate the adaptive immune system. Other cell types activated by imiquimod include NK cells, macrophages, and B-lymphocytes [18,27].

In this retrospective study, we sought to determine the efficacy and toxicity of self-administered topical 5% imiquimod cream for the treatment of persistent HPV infections either with or without CIN/VAIN involvement.

Patients and methods

This retrospective study was approved by the Institutional Review Board (IRB) of the Chang Gung Memorial Hospital. Patients with persistent HPV infection (≥1 year) after a history of treatment for cervical or vaginal neoplasms (Group 1), abnormal Papanicolaou (Pap) smears without abnormal histology (Group 2), and untreated histology-documented CIN/VAIN Grade 1/2/3 (Group 3) with HPV-positive testing were recruited. Abnormal Pap smears were defined as those containing atypical squamous cells of undetermined significance (ASCUS) or worse. The participants were informed about the treatment and possible complications and accepted the off-label use of imiquimod (Aldara; 3M Health Care Limited, Leicester, UK). Patients were instructed to apply 250 mg of 5% imiquimod cream intravaginally, using their fingers to reach the exocervix or vaginal vault, every other day or at least twice weekly on an outpatient basis for a minimum of 12 doses.

HPV DNA testing primarily used a Hybrid Capture II method (Digene Corporation, Gaithersburg, MD, USA), which detects 13 different carcinogenic HPV types. The procedures for collecting and transporting the specimens were conducted according to the manufacturer's instructions [6]. A positive cutoff value was set at 1 pg HPV DNA per mL in the specimen. HPV detection using SPF1/GP6+ polymerase chain reaction plus genotyping with Easychip HPV Blot (King Car, I-Lan, Taiwan) was performed in selected patients [28,29].

The clinical outcomes were classified as progressive, stable disease, or complete regression. Colposcopy and cytology were usually performed at each visit when histology/cytology abnormalities remained or HPV testing was persistently positive. Complete regression criteria were satisfied only if cytology/colposcopy results were normal and HPV tests were negative. Progression was defined by cytologic or histological progression of ≥1 grade and persistently positive HPV. All others were considered to be a stable disease outcome (such as cytologic improvement that was not accompanied by reversion to a normal Pap smear or persistent positive HPV testing or Pap of ASCUS with negative HPV test). HPV follow-up status was classified as either positive or negative. The duration of response was required to last for at least 6 months to be declared a success.

A group of age- and previous diagnosis-matched, untreated historical controls of Group 1 were selected for comparison from our ongoing follow-up cohorts. These controls had normal Pap and colposcopy/histology results but were persistently HPV-positive for at least 1 year were eligible if they had a follow-up HPV test at least 6 months following the second HPV-positive test and: (1) a previous history of CIN2/3 with hysterectomy (grant CMRPG 371641); (2) a previous history of CIN2/3 without hysterectomy (grant CMRPG380731); (3) a baseline normal cytology but positive HPV tests (grant NSC97-2314-B-182-013-MY3); or (4) a history of abnormal Pap results or cervical neoplasia that did not fit into the above prospective studies.

All calculations were performed using the SPSS 15.0 statistical package (SPSS, Inc., Chicago, IL, USA). Associations between categorical variables were assessed using the Chi-square statistical analysis. Comparisons between the historical control group and the imiquimod treatment group were performed using the Kaplan-Meier method (log-rank test). A two-sided -p-value < 0.05 was considered to be statistically significant.

Results

Between February 2003 and July 2007, 72 patients were eligible for analysis (Table 1). Based on the histology and cytology results upon entry into treatment, the study patients were grouped as follows: Group 1 ($n = 26$), patients with persistent HPV infection (≥1 year) but normal histology and cytology; Group 2 ($n = 20$), patients with abnormal Pap tests but negative histology (9 ASCUS, 2 atypical squamous cells, cannot rule out a high grade lesion, 8 low-grade squamous intraepithelial lesions, and 1 high-grade squamous intraepithelial lesion); and Group 3 ($n = 26$), patients with histological diagnoses of CIN/VAIN Grade 1/2/3 (20 CIN1/VAIN1, 3 CIN2/ VAIN2, 2 VAIN3, and 1 CIN3).

The median age of the study population was 54 years (range 26–88 years). Ten patients had previously been diagnosed with cervical cancer (5 radical hysterectomies and 5 radiation/concurrent chemoradiation treatments), 29 patients had prior CIN/VAIN 2/3 diagnoses (10 hysterectomies, 18 conizations, and 1 biopsy), 29 patients had prior CIN1/VAIN1 diagnoses (9 conizations, 17 biopsies, 1 partial vaginectomy, and 2 laser therapies), two patients had ASCUS, one patient had endometrial cancer (abnormal vaginal Pap test at post-therapy surveillance), and one patient underwent a hysterectomy for adenomyosis (symptomatic with an abnormal vaginal Pap smear). The clinical profile of the historical control group is also summarized in Table 1.

The first HPV test for the eligible patients occurred 3–6 months from the initiation of imiquimod treatment. Overall, 38 women (52.8%) had HPV regression, and 37 women (51.4%) were both HPV-negative and had normal cytological and histological findings at a median follow-up of 33.6 months. Six patients (8.3%) had lesions that progressed and persistent HPV infections (Table 2). Additionally, 20 of 34 patients (58.8%) with persistent HPV infection also had complete regression in their cytology/histology results. Complete regression was achieved in 17 of 26 patients (65.4%) in Group 1. Of the 20 women in Group 2, nine (45%) reverted to HPV-negative status with normal cytologic findings following treatment with imiquimod. In Group 3, 11 women (42.3%) reverted to HPV-negative status.

The side effects of imiquimod treatment were acceptable (Table 3); no participant discontinued medication due to adverse effects in the present study. The most frequent side effect was increased vaginal discharge. Other side effects of imiquimod included vulvar pain, pruritus, myalgias, and flu-like symptoms. The investigators also noted skin reactions, including erythema and erosions.

To examine the efficacy of imiquimod treatment, the complete regression rate of Group 1 imiquimod-treated patients was compared with that of individuals in the historical control group. Of the 20 matched controls who had a follow-up HPV test at least 6 months following the second HPV-positive test, six (30.0%) had HPV clearance without developing new Pap or histological abnormalities. There was a significant difference in the clearance rates between the imiquimod-treated patients and the historical control individuals in Group 1 ($p = 0.036$, log-rank test). In Group 1, the mean time of HPV clearance was 13.2 months (complete regression in 16 of 17 patients occurred within 24 months), which was much faster than that observed in the historical control group (36.2 months, Fig. 1).

Discussion

The clinical utility of the integration of HPV detection in cytology in the follow-up of women treated for CIN has been confirmed [29]. However, persistent HPV infection is quite prevalent in women who suffered from prior CIN lesions [6]. We retrospectively analyzed 72 patients who underwent self-administered imiquimod treatment. An HPV regression and normal cytology/histology rate of 51.4% over a median follow-up time of 33.6 months was found within the entire patient cohort. Of the 26 patients who had a normal Pap test but were persistently HPV positive for at least 1 year (Group 1), there was a regression rate of 65.4%, which was significantly different from the rate observed for the untreated historical control group (30%, $n = 20$; $p = 0.036$). Unfortunately, women with a concomitant abnormal cytology (Group 2) or CIN or VAIN 1/2/3 diagnosis (Group 3) appeared to be less likely to regress, exhibiting regression rates of 40% and 46.1%, respectively.

A recent review of the VAIN response to imiquimod treatment [30,31] demonstrated that the complete response rates ranged from 50% [25] to 86% [23, 26], and the partial response rates ranged from 14% [26] to 25% [25]. Buck et al [23] noted that 86% of the 42 women available for follow-up (mostly low-grade VAIN) showed clearance upon colposcopic evaluation after self-administering imiquimod treatment once weekly. Diaz-Arrastia et al [25] also treated patients with self-

Table 1
Characteristics of imiquimod-treated groups and the historical control group.

	Group 1 ($n = 26$)	Group 2 ($n = 20$)	Group 3 ($n = 26$)	Entire cohort ($n = 72$)	Historical control group ($n = 20$)
Median age, y (range)	54 (32–80)	48.5 (35–75)	54 (26–88)	53.5 (26–88)	50 (31–77)
Past history					
ASCUS	0	2	0	2	0
CIN1	10	6	13	29	8
CIN2/3	12	7	10	29	12
Cervical cancer	4	3	3	10	0
Endometrial cancer	0	1	0	1	0
Adenomyosis	0	1	0	1	0

ASCUS = atypical squamous cells of undetermined significance; CIN = cervical intraepithelial neoplasia.

Table 2
Cytological and human papillomavirus (HPV) changes following imiquimod application based on pretreatment cytology, histology, and group ($n = 72$).

Pretreatment cytology and histology (number of patients)		Posttreatment outcomes, *n* (%)				
		Stable disease		Complete regression	Progression	
		HPV+	HPV−	HPV−	HPV+	HPV−
Pre-treatment cytology						
Normal (32)		9 (28.1)	1 (3.1)	**20 (62.5)**	2 (6.3)	0
LSIL (19)		9 (47.4)	0	**9 (47.4)**	1 (5.3)	0
HSIL (2)		1 (50)	0	**0**	1 (50)	0
ASCUS (16)		7 (43.7)	0	**7 (43.7)**	2 (12.5)	0
ASC-H (3)		2 (66.7)	0	**1 (33.3)**	0	0
Pretreatment histology						
Group 1	Cytology nl, colposcopy nl (24)	6 (25)	1 (4.2)	**15 (62.5)**	2 (8.3)[b]	0
	Cytology nl, colposcopy abnl (2)[a]	0	0	**2 (100)**	0	0
Group 2	Cytology abnl, bx nl (7)	4 (57.1)	0	**2 (28.6)**	1 (14.3)	0
	Cytology abnl, colposcopy nl (13)	5 (38.5)	0	**7 (53.8)**	1 (7.7)	0
Group 3	CIN1/VAIN1 (20)	12 (60)	0	**7 (35)**	1 (5)	0
	CIN2,3/ VAIN2,3 (6)	1	0	**4 (66.6)**	1 (16.7)	0

Abnl = abnormal; ASCUS = atypical squamous cells of undetermined significance; ASC-H = atypical squamous cells − cannot exclude high-grade lesions; bx = biopsy, CIN = cervical intraepithelial neoplasia; HSIL = high-grade squamous intraepithelial lesion; LSIL = low-grade squamous intraepithelial lesion; nl = normal; VAIN = vaginal intraepithelial neoplasia.

[a] Biopsy results were negative; [b] One patient progressed to CIN2 at 16 months and another to CIN1 in histology at 15 months after imiquimod treatment. The patients who had cytologic/histologic regression and tested HPV-negative are denoted in bold text.

administered imiquimod ($n = 7$); two of these patients had high-grade CIN, and two patients had high-grade VAIN. Four patients exhibited a complete response to treatment, two patients exhibited a partial response to treatment, and one patient progressed despite treatment. Of the four complete responders, two patients remained disease-free (mean follow-up: 33 months). Haidopoulos et al [26] reported that six of seven patients with VAIN2/3 had vaginal dysplasia that regressed by at least two grades in response to physician-administered imiquimod therapy. In contrast, only 12 (46.1%) had HPV reversion to negative status in Group 3 of our study cohort. However, none of these previous studies required the complete clearance of HPV infection at the primary end point, which results in different success rates between our study and other reports. Additionally, our study is the largest thus far that has examined the use of intravaginal imiquimod therapy.

In terms of anatomic accessibility by topical application, imiquimod is better suited for VIN patients. For treating lesions in the vagina or cervix, the self-application of topical imiquimod has disadvantages. However, we postulate that HPV infection in the female genital tract could be multifocal, and an enhanced immune response could be widespread. Thus, an accurate application to the lesion site may not be critical. Moreover, this application method is more convenient for the patient if it achieves the desired results.

There were limitations to the current study. This was a retrospective study with a small sample size. Additionally, most of the cases in the study cohort were analyzed using the Hybrid Capture II HPV testing. HPV genotyping was performed in a small number of the participants, and we classified the patient's HPV status as positive or negative regardless of the type-specific persistence or whether it was a newly acquired infection.

Table 3
Side effects of imiquimod treatment.

Side effects	Number of patients
Indicated by the patient	
Vulvar pain	2
Vulvar pruritus	3
Flu-like syndrome	2
Muscular ache	1
Increased vaginal discharge	7
Indicated by the physician	
Mild erosion	2
Mild erythema	1
Fever	3

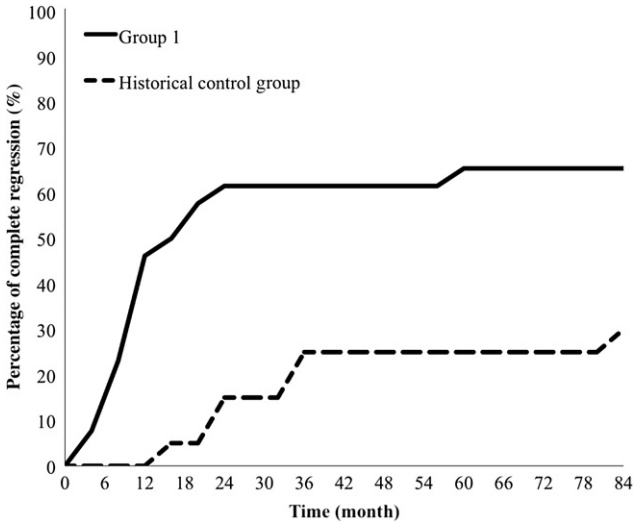

Fig. 1. Cumulative clearance curves for subjects in Group 1 and the historical control group ($p = 0.036$).

The clinical value of treating persistent HPV infection with normal cytology remains controversial. With the wide application of HPV primary screening, a large number of women may experience substantial anxiety knowing that they are carrying a persistent, high-risk HPV infection in their lower genital tracts. Intravaginal imiquimod treatment may be a viable option for these women if it is proven effective by well-designed placebo-controlled, randomized trials. If selecting Group 2 or 3 patients, a sample size of 170 patients in each arm would be required to achieve 80% power for the detection of a 15% improvement in the treatment arm versus a spontaneous regression rate of approximately 30% in the placebo group using a two-sided χ^2 test. A much smaller sample size would be necessary for Group 1 patients.

In conclusion, 51.4% of patients had cytologic/histologic regression and tested HPV-negative after treatment with 12 × 250 mg doses of intravaginal, self-administered imiquimod. The rates of HPV clearance (69.2%) in women who had a normal Pap test but were persistently HPV-positive for at least 1 year were significantly different from those of the untreated historical control group (30%). The tolerability of intravaginal, self-administered imiquimod has been confirmed. Its efficacy in the treatment of women with persistent HPV infection and normal cytology warrants further randomized, controlled trials to determine appropriate dosages and scheduling.

Declaration of ethical approval

The Institutional Review Board (IRB) of the Chang Gung Memorial Hospital approved this retrospective study on July 12, 2007 (IRB no. 96-0624B).

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